

Natural Habitat of *Cryptococcus neoformans* var. *gattii*

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Environmental isolations have established that *Cryptococcus neoformans* var. *gattii* appears to have a specific ecological association with *Eucalyptus camaldulensis*. So far, we have isolated *C. neoformans* var. *gattii* on 35 separate occasions, all from samples associated with *E. camaldulensis*. The global distribution of *E. camaldulensis* appears to correspond to the epidemiologic distribution of cryptococcosis caused by *C. neoformans* var. *gattii*. No other environmental source for the fungus has yet been detected, and no other eucalypt has the distribution pattern corresponding to reported cases caused by this fungus. These findings may provide an explanation for the high incidence of infections caused by *C. neoformans* var. *gattii* in Australian aborigines living in the Northern Territory and for its low worldwide incidence in acquired immunodeficiency syndrome patients.

Cryptococcus neoformans has a worldwide distribution and exhibits antigenic variability, with four serotypes (A, B, C, and D) being recognized (11). Important differences between the AD and BC serotype pairs have been demonstrated, and *C. neoformans* has been subdivided into two varieties: *C. neoformans* var. *neoformans* (serotypes A and D) and *C. neoformans* var. *gattii* (serotypes B and C) (7). The major environmental source of *C. neoformans* var. *neoformans* throughout the world has been bird excreta, whereas the natural habitat of *C. neoformans* var. *gattii* has remained unknown. There is evidence from patient isolates that *C. neoformans* var. *gattii* is worldwide in scope, corresponding to a subtropical to tropical climate (5). Furthermore, a recent survey of the incidence of *C. neoformans* varieties among clinical isolates revealed that rural areas of South Australia and the Northern Territory are endemic regions for the occurrence of *C. neoformans* var. *gattii* (4). Therefore, a search for the natural habitat of *C. neoformans* var. *gattii* was commenced in the Barossa Valley, the nearest rural area to Adelaide with a recently recorded case of disseminated cryptococcosis caused by this fungus.

This report identifies the first reported natural habitat of *C. neoformans* var. *gattii* and proposes an explanation for its (i) worldwide geographic distribution, (ii) high incidence in Australian aborigines, and (iii) low incidence in acquired immunodeficiency syndrome (AIDS) patients.

Large-scale sampling of air, soil, and vegetation from the Barossa Valley commenced in April 1989. In order to cover all seasonal variations, selected sites were sampled on a weekly basis. Air samples (180 liters/min for 5 min) were collected directly onto *Guizotia abyssinica* creatinine agar (GACA) (10) plates by using an SAS model 5203 sampler manufactured by Pool Bioanalysis Italiana. Collected soil and vegetation samples were shaken in 20 ml of sterile distilled water and allowed to stand for 10 to 15 min, and then 0.5-ml samples of the resulting suspensions were streaked onto GACA and incubated at 26°C for 7 days. Cultures were examined daily, and the identities of all yeast colonies exhibiting the brown color effect on GACA were determined by using a battery of biochemical, physiological, and morphological tests, including fermentation and assimilation studies (1). Canavanine-glycine-bromthymol blue agar

(7) was used to distinguish between *C. neoformans* var. *neoformans* (A and D serotype group) and *C. neoformans* var. *gattii* (B and C serotype group).

In November 1989, after an 8-month sampling period during which 2,100 collections were made, *C. neoformans* var. *gattii* was isolated simultaneously from two sites in the Barossa Valley. The first site was at the Barossa Reservoir, situated 5 km south of Sandy Creek, while the second site was in parklands at Nuriootpa, 25 km to the north. At both sites, *C. neoformans* var. *gattii* was initially isolated from plant debris collected from under the canopies of *Eucalyptus camaldulensis* trees (Fig. 1). Extensive collections were then made from all vegetation at these sites over a 4-week period. During this time, *C. neoformans* var. *gattii* was repeatedly isolated, but only from material associated with *E. camaldulensis*, including wood, bark, leaves, and accumulated debris lying under the canopies of the trees. All other plant materials and soils collected from these sites were negative, indicating a specific association between *C. neoformans* var. *gattii* and *E. camaldulensis*.

It was observed that the sudden appearance of *C. neoformans* var. *gattii* in the environment appeared to coincide with flowering of the *E. camaldulensis* trees in the area under study. Air-sampling experiments conducted under the canopy of an *E. camaldulensis* tree in flower have detected the presence of airborne propagules of the fungus. This suggests that dispersal of *C. neoformans* var. *gattii* may occur in late spring concomitant with the flowering of its natural host. All other air-sampling experiments, including those conducted under *E. camaldulensis* trees not in flower, have so far failed to detect *C. neoformans* var. *gattii*.

In order to substantiate our findings, collections were made from *E. camaldulensis* trees growing along the Murrumbidgee River at Balranald and Hay, two rural towns situated in southwestern New South Wales, approximately 500 km away from the Barossa Valley sites. *C. neoformans* var. *gattii* was isolated from both areas, once again from bark and accumulated debris at the bases of the trees. So far, we have isolated *C. neoformans* var. *gattii* on 35 separate occasions, all from samples associated with *E. camaldulensis*.

The geographic distribution of *E. camaldulensis* in Australia (3) (Fig. 2) appears to correspond to the epidemiologic distribution of patient isolates of *C. neoformans* var. *gattii* (4), with endemic foci around Darwin, Alice Springs, rural

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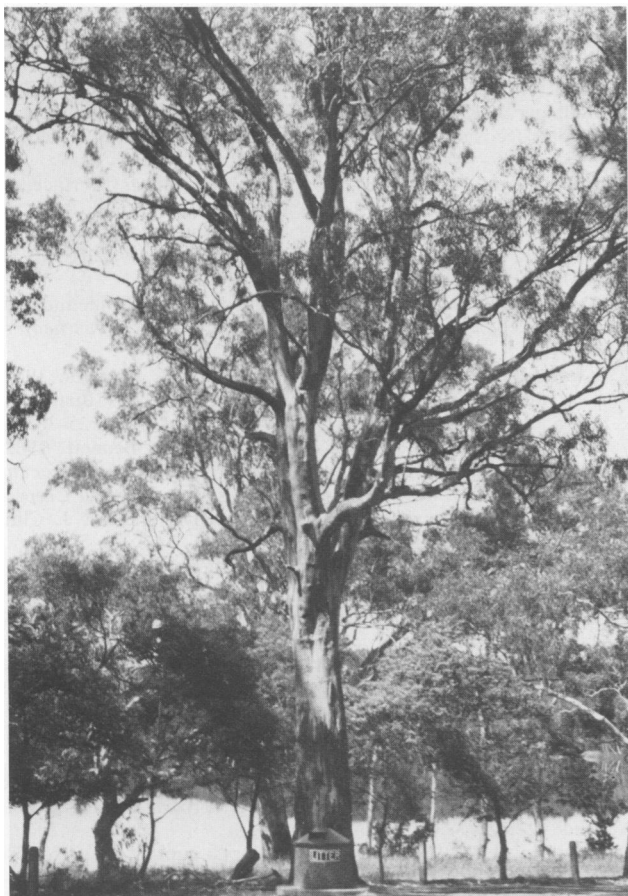


FIG. 1. *E. camaldulensis* at the Barossa Reservoir site.

areas of South Australia, Victoria, New South Wales, Queensland, and Western Australia. *E. camaldulensis* does not occur in Tasmania, nor, to our knowledge, do *C. neoformans* var. *gattii* infections. No other *Eucalyptus* species has this distribution pattern (3).

The river red gum or *E. camaldulensis* has been exported

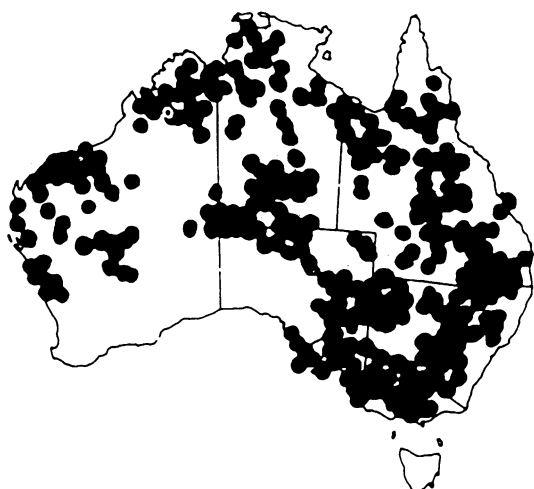


FIG. 2. Distribution map of *E. camaldulensis* in Australia (3) (reproduced by permission).

extensively from Australia, and it is the second most widely planted eucalypt throughout the world after *E. globulus* (the Tasmanian blue gum) (12). The name was bestowed by Frederick Dehnhardt, the chief gardener and curator of the gardens of the Count of Camalduli, a region near Naples, Italy. Dehnhardt described a specimen of this tree growing there as an exotic in 1829. In Australia, it is the most widely distributed of all eucalypts, occurring in all states except Tasmania. *E. camaldulensis* has also been reported as growing in the following countries: Spain, Portugal, Italy, Cyprus, Greece, Malta, Turkey, Israel, Iraq, Saudi Arabia, Jordan, India, Sri Lanka, Nepal, Pakistan, the USSR, the People's Republic of China, Japan, Algeria, Libya, Tunisia, Morocco, South Africa, Lesotho, Zimbabwe, Ethiopia, Sudan, Kenya, Uganda, Tanzania, Nigeria, Angola, Ghana, Ivory Coast, Sierra Leone, Chad, Niger, Zaire, the Malagasy Republic, Mauritius, Comoro Islands, the United States (especially in Hawaii and California and, to a lesser extent, in Arizona, New Mexico, Texas, and Florida), Mexico, Argentina, Brazil, Peru, Uruguay, Paraguay, Colombia, Guyana, and New Zealand (9, 12).

In general, eucalypts do not grow readily outdoors in most of Europe, and it is only in limited areas of the Mediterranean littoral that there are well-established plantation trees. None thrive north of the Pyrenees. Growth outside a band extending from 45°N to 45°S is quite unusual, and there is always some climatic explanation for vigorous growth outside these latitudes (12). This corresponds to the observations of Kwon-Chung and Bennett (5), who concluded from patient isolates that *C. neoformans* var. *gattii* was prevalent only in tropical and subtropical regions, particularly in southern California, Brazil, southern Asia, and central Africa.

The need for exposure to *E. camaldulensis* trees, probably in late spring, in order to acquire an infection by *C. neoformans* var. *gattii* also provides a plausible explanation for the high incidence of infections caused by this fungus in Australian aborigines living in the Northern Territory and for its low worldwide incidence in AIDS patients.

The high incidence of cryptococcosis in the aboriginal population of the Northern Territory is well documented (3, 4). It has already been suggested that the lifestyle of the aboriginal population in the Northern Territory may be a significant factor, in that all the patients came from settlements, missions, cattle stations, or "out bush"; none came from the major towns. *E. camaldulensis* grows extensively along the watercourses, flood plains, and alluvial flats with a high water table (12). This is the primary area inhabited by aborigines who live in close association with *E. camaldulensis* trees; therefore, they have a high exposure to *C. neoformans* var. *gattii*. This fungus-eucalypt association would also explain earlier reports in the literature of cryptococcosis occurring in the koala (2), which frequently inhabits *E. camaldulensis* trees.

Only two cases of *C. neoformans* var. *gattii* infections have been reported from AIDS patients (K. J. Kwon-Chung and A. Varma, Abstr. 3rd Symp. Topics Mycol., Mycoses AIDS Patients, p. 55-56, 1989), one from a Zairean patient and the other from a Canadian patient who had been to Mexico before acquiring his infection. We believe that AIDS patients are not being exposed to *C. neoformans* var. *gattii* because they are not in regular contact with *E. camaldulensis* trees. It is noteworthy that *E. camaldulensis* does not occur naturally along the eastern coastal areas of Queensland, New South Wales, and Victoria (3), where 90% of Australian AIDS patients live (National Health and Medical

Research Council Special Unit in AIDS Epidemiology and Clinical Research, 27 December 1989). As mentioned above, both Zaire and Mexico have recorded plantations of *E. camaldulensis* (9, 12).

In conclusion, environmental isolations have indicated a specific association between *C. neoformans* var. *gattii* and *E. camaldulensis*. No other environmental source for *C. neoformans* var. *gattii* has yet been detected. Dispersal of *C. neoformans* var. *gattii* appears to occur in late spring, concomitant with the flowering of *E. camaldulensis*. Further experiments are in progress to determine the precise timing and duration of spore release; however, we suspect that airborne infectious propagules of the fungus may only be present in the environment for relatively short periods. The global distribution of *E. camaldulensis* appears to correspond to the distribution of cryptococcosis caused by *C. neoformans* var. *gattii*. Our data indicate that *C. neoformans* var. *gattii* may be similar to some of the smut fungi that are host specific to angiosperms. Studies of the host-parasite interaction between *C. neoformans* var. *gattii* and *E. camaldulensis* are also in progress. From the results reported in this paper, we suggest that the natural habitat of *C. neoformans* var. *neoformans* may also be a specific angiosperm. It is possible that bird excreta is simply a unique environmental niche which acts as a vector for the dispersal of this variety of the fungus.

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